# *Enhancing Crop Productivity Through Increased Abiotic-Stress Tolerance and Biomass Production*

Malcolm D. Devine
*Performance Plants Inc.*
*Saskatoon, Saskatchewan*

Crop yields are limited by a combination of biotic stresses, abiotic stresses, and nutritional factors. Various analyses have suggested that abiotic stress—due primarily to drought, heat, cold or salinity—is the major factor that prevents crops from realizing their full yield potential (*e.g.* Boyer, 1982; Edmeades, 2008). Increases in crop yields through conventional plant breeding result from genetic enrichment by introducing multiple quantitative trait loci (QTLs), including, presumably, QTLs for abiotic and biotic stress resistance. For example, Tollenaar and Wu (1999) have provided evidence that yield increases in corn (*Zea mays* L.) in recent decades have been partly due to enhanced stress tolerance during the grain filling and late-maturation stages.

Separately, recent interest in renewable fuels has led to a substantial increase in ethanol production from plant material. Although the initial emphasis has been on using starch from corn, and to a lesser extent from other food grains, this is unlikely to be sustainable in the long term; alternatives such as cellulose from specially grown "biomass" crops are likely to be a more important substrate for ethanol production. The challenge here is to increase total vegetative biomass rather than seed yield, similar to the approach one might take in forage or silage-crop improvement.

This paper describers several examples of how modern crop technologies, in particular transgenic approaches, may be applied to broaden crop tolerance of various abiotic stresses and to increase total biomass in non-seed crops.

## Drought Tolerance

Drought limits crop yields in many parts of the world, and research has identified many genes that may contribute to enhancing plant performance under drought stress. In a recent study, seven independent genes previously shown to confer some tolerance of

drought were compared in a transgenic rice study (Xiao *et al.*, 2008). The genes included transcription factors (*e.g. CBF3*), genes involved in abscisic acid (ABA) biosynthesis (*e.g. NCED2*, *LOS5*) and genes involved in oxygen-radical detoxification (*e.g. SOS2*). Many of the transgenic plants carrying these genes yielded more than the wild-type rice under drought conditions. The authors emphasized the importance of evaluating transgenics under both stressed and non-stressed conditions in the field to obtain a full understanding of gene performance.

In a separate study, over-expression of the transcription factor Nuclear Factor Y B subunit conferred protection against drought in *Arabidopsis thaliana* (L.) Heynh. and corn (Nelson *et al.*, 2007). Drought tolerance has also been conferred by expression of a gene for isopentenyl transferase involved in cytokinin biosynthesis (Rivero *et al.*, 2007).

At Performance Plants, research on drought stress has been focused on a key step in the ABA-signaling pathway. It was initially observed that a knock-out of the farnesyl transferase β subunit (FTB) protected *Arabidopsis* plants against drought; however, the knock-out plants exhibited some deleterious effects that delayed flowering and reduced yield (Pei *et al.*, 1998). Subsequently it was shown that down-regulation of FTB in *Arabidopsis*, using either anti-sense or RNA interference, resulted in a drought-tolerant phenotype without the negative effects of the full knock-out (Wang *et al.*, 2005). The primary physiological mechanism underlying this response is enhanced sensitivity to the ABA signal produced under drought-stress conditions, resulting in more-rapid stomatal closure, increased water retention in the plant, and increased seed yield (Figure 1).

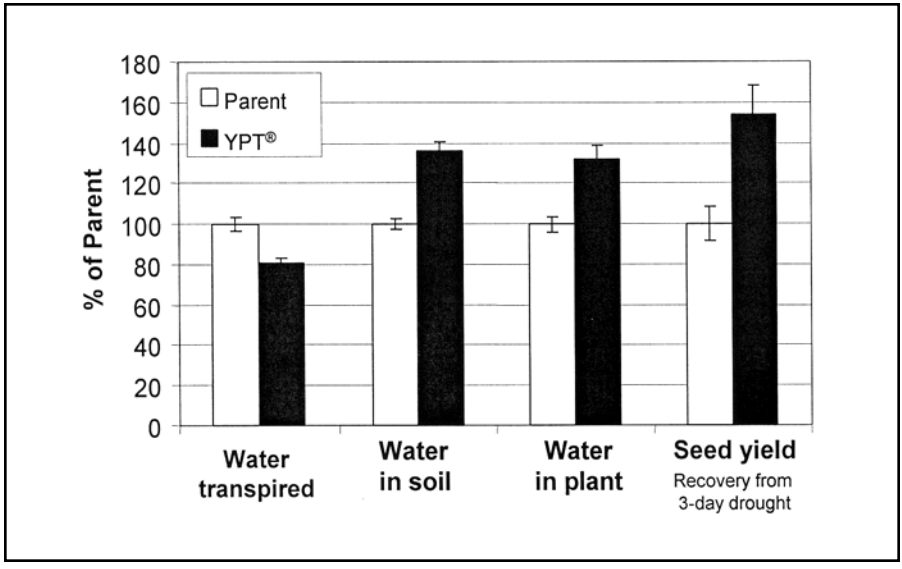


Figure 1. Leaf transpiration, soil-water content, plant-water content and seed yield in *Arabidopsis*, conferred by down-regulation of farnesyl transferase β (YPT®). Data are expressed as percent of the parent (wild-type) control (Pei *et al.*, 1998).

This research was subsequently extended to the farnesyl transferase α subunit (FTA; the functional farnesyl transferase protein is a heterodimer of the α and β subunits, both of which are required for enzyme activity). Down-regulation of FTA also resulted in a drought-tolerant phenotype in *Arabidopsis* (Wang *et al.*, 2009).

Down-regulation of FTB or FTA in canola (*Brassica napus* L.) has been shown to confer protection to plants growing in the field over several growing seasons in western Canada (Wang *et al.*, 2005; Wang *et al.*, 2009). Under well-watered conditions there was no yield drag and all agronomic and seed-quality parameters were similar in the transgenic and wild-type plants. In one field experiment, the yield of wild-type canola was significantly reduced under limited irrigation conditions, whereas the yield of two independent transgenic lines with down-regulated FTB activity was not affected under these conditions (Figure 2). Overall, yield increases of up to 26% over the wild-type have been observed in transgenic canola growing under dryland conditions in western Canada.

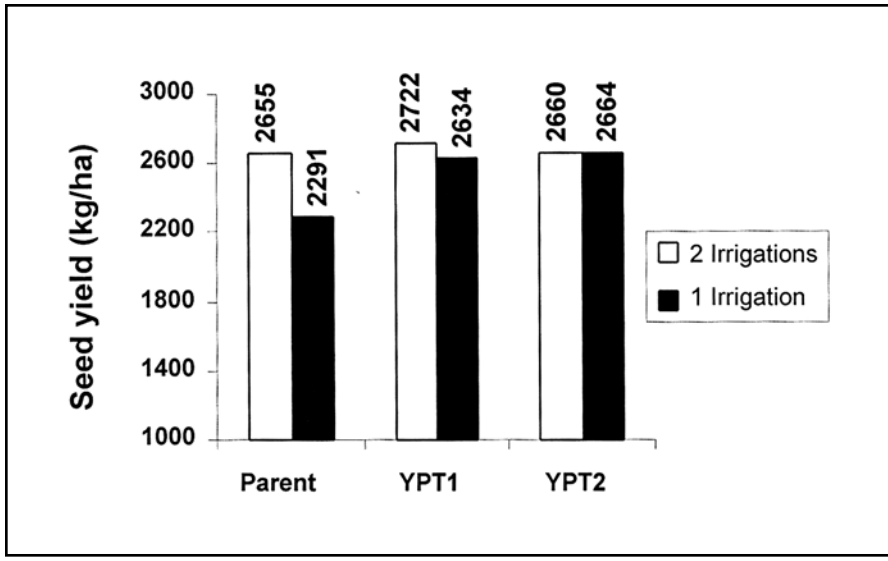


Figure 2. Seed yield of parent (wild-type) canola and two independent FTB down-regulation lines (YPT1 and YPT2) grown under well-watered and reduced-irrigation conditions. Irrigation was applied twice (=well-watered) or only once during the flowering period. The yield of the FTB down-regulation lines under one irrigation was significantly higher than that of the parent under similar conditions (Wang *et al.*, 2005).

The ABA-signaling pathway resulting in stomatal closure is highly conserved in higher plants. This approach of down-regulating FTA/FTB is currently being extended to several other important crops, with the aim of protecting their yields under water-limiting conditions.

## Heat Tolerance

Recent concerns about global warming have stimulated interest in the effects of high temperatures on crop production and crop yields. In light of the anticipated temperature increases, acquisition of thermotolerance is seen as a key strategic target in maintaining crop yields (Wahid *et al.*, 2007). Various analyses (*e.g.* Battisti and Naylor, 2009; Lobell and Asner, 2003) suggest that increasing temperatures will pose a major constraint to crop production in the future. A recent study suggested that the warmest summers observed in the tropics and subtropics in the past century may be seen as normal by the end of the 21st century (Battisti and Naylor, 2009).

Existing data already illustrate quite clearly that high temperatures frequently limit crop-seed yields. Field-grown canola yields were significantly reduced by temperatures of ≥30°C during flowering. The yield of three *Brassica* species, *B. napus*, *B. rapa* and *B. juncea*, were all reduced by high-temperature stress, although reductions were less in *B. rapa* and *B. juncea* than in *B. napus* (Angadi *et al.*, 2000). Recent observations in the prairie provinces of Canada suggest that canola yields are lower in summers in which high temperatures occur during flowering.

A recent retrospective analysis of rice yields at the International Rice Research Institute in the Philippines indicated that increasing temperature was correlated with a reduction in rice yield. The reduced rice yields were correlated with higher night temperatures but not with daytime temperature increases, which were only about one third as high as the increase in night temperature maxima (Peng *et al.*, 2004). Each increase of 1°C in the night-time maximum temperature was associated with a 10% decrease in rice yield. This study highlights the importance of studying the independent effects of increases in day and night temperatures to understand the underlying physiological basis for high-temperature effects on crop growth and yield.

At Performance Plants we have undertaken a large-scale screening program to identify candidate genes associated with heat tolerance. Several have been identified and confirmed as conferring heat tolerance in *Arabidopsis*. Some of these same genes, or their *Brassica* homologs, linked to an appropriate promoter, confer heat tolerance in flowering canola plants (Figure 3). A selection of these putative heat-tolerant lines will be field-tested for the first time in 2009.

## Other Abiotic Stress-Tolerance Traits

Although not reviewed in this paper, cold and freezing tolerance has received as much attention as drought over the years. Substantial improvements have been made in low-temperature tolerance through conventional breeding, more recently enhanced by the use of molecular markers associated with the trait. Although many genes have been identified as putative cold-tolerance genes, it would appear that none of these has yet demonstrated sufficient protection to merit commercialization in large-scale crop production. However, despite the trends of higher temperatures in many regions, protection against the devastating effects of low temperatures, particularly during the sensitive phases of seedling growth and crop maturation, remains an important focus area for crop improvement.

Figure 3. Canola (*Brassica napus*) plants subjected to heat stress during flowering. Left, plant containing transgene for heat tolerance; right, wild-type. (Photo courtesy of Performance Plants Inc.)

Water-use efficiency (WUE) has some elements in common with drought tolerance, in that it can be seen as a mechanism whereby a plant can avoid drought by depleting soil moisture more slowly. However, the physiological mechanisms of the two traits are likely different, as is their place in agricultural cropping systems. WUE is being recognized as a critically important trait in areas where crop production relies on dwindling supplies of sub-surface irrigation water or where there is competition for water between urban and agricultural demands.

Approaches to enhancing WUE center on altering the ratio of $CO_2$ assimilated per unit of water transpired (Chaerle *et al.*, 2005). In one study, an *Arabidopsis* transcription factor, *HRD*, was shown to increase WUE in rice (Karaba *et al.*, 2007). The transgenic plants in this study exhibited higher shoot biomass under well irrigated conditions and more root biomass under drought stress. WUE was also linked to changes in leaf morphology and mesophyll-cell structure that contributed to higher rates of photosynthesis. It is very likely that we will see considerably more effort in this area of research in the coming years as concerns over water availability increase.

## Stress Combinations

Abiotic stresses often occur in combination. For example, heat and drought stress frequently occur simultaneously. Mittler (2006) has emphasized the importance of studying plant responses to combinations of stresses rather than stresses in isolation, since a plant's response and coping mechanism may differ according to which stresses or combination of stresses it is experiencing.

Some stress-protection mechanisms appear to confer tolerance of multiple stresses, for example through effects on energy balance or detoxification of reactive oxygen species generated upon exposure to stress. Down-regulation of poly(ADP-ribose) polymerase (PARP) in *Arabidopsis* and canola increased tolerance of heat, drought and high light (De Block *et al.*, 2004). While this was originally believed to be due to reduced $NAD^+$ depletion and reduced ATP consumption, subsequent research indicated that reducing PARP expression also induced ABA production, which, in turn, led to induction of a wider range of stress-tolerance genes (Vanderauwera *et al.*, 2007; Metzlaff, this volume[1]). This may provide a common mechanism of tolerance of multiple stresses. In a similar vein, different stresses that result in oxygen-radical production (*e.g.* ozone, high temperature) may be mitigated by common mechanisms that detoxify these radicals.

Clearly, stress tolerance is complex and no single approach can provide a solution to the multiple environmental stresses that a plant might experience during its lifecycle. Further evaluation of novel germplasm and putative stress-related genes under field conditions will be required to develop a robust set of new germplasm better adapted to withstand the stresses a plant may be exposed to.

## Enhanced Biomass Production

As mentioned above, there is increasing interest in production of plant biomass for cellulosic ethanol production. A wide range of plant species, monocots and dicots, annuals and perennials, and herbaceous and woody plants, is currently being evaluated for total biomass production. For the business of ethanol production from biomass to be viable, a certain minimum productivity per unit land area will be required to keep substrate costs low.

In addition to selecting and breeding for high biomass-yielding varieties, some effort is now going into the use of transgenic approaches to increase total plant biomass. At Performance Plants we have identified a novel allele of a gene involved in the transition from vegetative growth to flowering. The effect of this allele is to delay flowering, thereby extending the period of vegetative growth. Plants carrying this trait have greater total biomass, larger leaves and thicker stems. Although flowering is delayed, the plants do eventually produce viable seed. This trait is now being incorporated into potential biomass crops for more extensive evaluation under field conditions.

[1]Pages 73–77.

## Conclusions

Crop production faces many challenges, due to changing environmental conditions and evolving needs for new plant-derived materials. No one approach will provide all the solutions. Rather, progress will be made by combining the existing approaches of breeding, enhanced by molecular markers linked to traits of interest, mining of novel alleles from germplasm collections, and introduction of novel alleles or variants of existing alleles from mutant populations. The path forward will require us to combine all possible approaches to maximize the chance of success in this important endeavor.

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**MALCOLM DEVINE** joined Performance Plants, Inc., in September 2006 as vice president for crop development and commercialization, bringing extensive experience in both public- and private-sector R&D in agriculture and plant biotechnology.

Dr. Devine obtained his training at the Universities of Glasgow (Scotland), Guelph, and Alberta. From 1987 to 1998 he held a faculty position in the Department of Plant Sciences at the University of Saskatchewan, serving as department head, 1994–1998. He then joined AgrEvo Canada (later to become Aventis CropScience Canada) as head of biotechnology research and spent 3 years in Ghent, Belgium, as head of technology acquisition and licensing for the BioScience division of Aventis CropScience, and later Bayer CropScience, with responsibility for technology evaluation, licensing and collaborations in support of BioScience research.

On his return to Canada in 2004, he spent 2 years as research director at the National Research Council Plant Biotechnology Institute in Saskatoon.